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Fine root demography in alfalfa (*Medicago sativa* L.)

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Abstract

In perennial forages like alfalfa (*Medicago sativa* L.), repeated herbage removal may alter root production and mortality which, in turn, could affect deposition of fixed N in soil. Our objective was to determine the extent and patterns of fine-diameter root production and loss during the year of alfalfa stand establishment. The experiment was conducted on a loamy sand soil (Udorthentic Haploboroll) in Minnesota, USA, using horizontally installed minirhizotrons placed directly under the seeded rows at 10, 20, and 40 cm depths in four replicate blocks. We seeded four alfalfa germplasms that differed in N_2 fixation capacity and root system architecture: Agate alfalfa, a winter hardy commercially-available cultivar; Ineffective Agate, which is a non-N_2-fixing near isoline of Agate; a new germplasm that has few fibrous roots and strong tap-rooted traits; and a new germplasm that has many fibrous roots and a strongly branched root system architecture. Video images collected biweekly throughout the initial growing season were processed using C-MAP-ROOTS software.

More than one-half of all fine roots in the upper 20 cm were produced during the first 7 weeks of growth. Root production was similar among germplasms, except that the highly fibrous, branch-rooted germplasm produced 29% more fine roots at 20 cm than other germplasms. In all germplasms, about 7% of the fine roots at each depth developed into secondarily thickened roots. By the end of the first growing season, greatest fine root mortality had occurred in the uppermost depth (48%), and least occurred at 40 cm (36%). Survival of contemporaneous root cohorts was not related to soil depth in a simple fashion, although all survivorship curves could be described using only five rates of exponential decline. There was a significant reduction in fine root mortality before the first herbage harvest, followed by a pronounced loss (average 22%) of fine roots at the 10- and 20-cm depths in the 2-week period following herbage removal. Median life spans of these early-season cohorts ranged from 58 to 131 days, based on fitted exponential equations. At all depths, fine roots produced in the 4 weeks before harvest (early- to mid-August) tended to have shorter median life spans than early-season cohorts. Similar patterns of fine root mortality did not occur at the second harvest. Germplasms differed in the pattern, but not the ultimate extent, of fine root mortality. Fine root turnover during the first year of alfalfa establishment in this experiment released an estimated 830 kg C ha^{-1} and 60 kg N ha^{-1}, with no differences due to N_2 fixation capacity or root system architecture.

Introduction

Alfalfa (*Medicago sativa* L.) roots are essential to productivity and persistence of the crop, but also promote accumulation of soil organic matter and organic N (Andrén et al., 1990). Annual inputs of C and N from decomposition of roots under continuous alfalfa have been estimated to be 1520 kg C ha^{-1} and 32 kg N ha^{-1} in Sweden (Andrén et al., 1991). During the establishment year on a silt loam soil in Minnesota, about 21 kg N ha^{-1} was released from decomposition of roots and nodules (Dubach and Russelle, 1994).

Growth and decomposition of fine roots occur simultaneously in a root system. Life spans of fine roots are highly variable and range from a few weeks to several years for trees and certain grass species (Vogt and Bloomfield, 1991). It is unknown whether fine

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roots have indeterminate life spans that are regulated by source-sink C partitioning or if fine roots have determinate life spans and die when they have fully metabolized a given supply of starch (Marshall and Waring, 1985; Vogt and Bloomfield, 1991). In either case, actual rates of fine root turnover (production, death, and decomposition) in the field are influenced by soil nutrient availability, cultural practices, species composition of the ecosystem, pathogen infestation, and soil faunal predation (Aber et al., 1985; Cheng et al., 1990; Joslin and Henderson, 1987; Nadelhoffer et al., 1985).

The shoot supplies carbohydrate to roots for growth, respiratory maintenance, and nutrient uptake (Lambers et al., 1983). Cultural practices that reduce the amount of C fixed by vegetative tissue, or that divert C from roots, can increase root senescence (Vogt and Bloomfield, 1991). In perennial forage species like alfalfa, vegetative regrowth following harvest appears to be dependent upon assimilates stored in the roots and crown (Hendershot and Volenec, 1993). After defoliation, new alfalfa shoots and crown buds are strong sinks for carbohydrates stored in the crown and taproot, which would slow root growth (Butler et al., 1959). The shoot becomes less dependent upon previously stored root reserves as photosynthetic capacity increases (Smith and Marten, 1970) and eventually root growth resumes, which suggests that there may be a cyclic pattern between root and shoot growth dependent upon the internal photosynthate status of the plant (Butler et al., 1959). For recently developed non-N_2-fixing alfalfas (Barnes et al., 1990), one might expect altered root growth and turnover because limited N supply would decrease shoot regrowth.

Root senescence in red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), and big trefoil (*Lotus uliginosus* Schk.) increases as a result of herbage removal by cutting (Butler et al., 1959; Wilson, 1942). Jones (1943) noted that net fine root growth of alfalfa was slow during summer and after harvest, but his observations were qualitative. Alfalfa root elongation rates become slower one day after harvest, and this effect lasted for 2 weeks, causing decreased rooting depth (Hodgkinson and Baas Becking, 1977). We found only limited evidence in the literature that total fibrous root mass in alfalfa declines after herbage cutting (Meyerhoff, 1981). Ta and Faris (1987) concluded that root turnover after harvest may help explain increased rates of N transfer from alfalfa to timothy (*Phleum pratense* L.), but did not measure root length changes.

Most work on fine root demography has concentrated on comparing plant species (Butler et al., 1959; Dubach and Russelle, 1994; McMichael et al., 1992; Meyer and Barrs, 1991) or environments (Andrén et al., 1993; Hansson et al., 1992; Hendrick and Pregitzer, 1993 ; Meyer and Barrs, 1991; Pregitzer et al., 1993), rather than on differences among plant genotypes or rootstocks (Kosola and Eissenstat, 1994). These latter researchers did not detect differences in fine root survival in dry topsoil of four citrus genotypes selected for different specific root length (cm g^{-1} root, Kosola and Eissenstat, 1994).

Root system architecture in most plants is thought to influence nutrient uptake and water-use efficiency (Fitter, 1991; Shein and Pachepsky, 1995), affects C partitioning between shoot and root (Nielsen et al., 1994), and may influence the extent of symbiotic N_2 fixation and winter hardiness in alfalfa (Johnson, 1992). Genetically-mediated expression of root system architecture can be influenced by soil type and condition, weather, cultural treatment, and mechanical and biotic plant injury (Carlson, 1925; McIntosh and Miller, 1981; Weaver, 1926).

Selection for alfalfa root traits such as lateral root number and fibrous root mass has been successful (Johnson, 1992). Recently, alfalfa germplasms (unique selections used in plant breeding programs) with different root system architectures have been produced via divergent phenotypic selection (Lamb et al., 1995a). The structural relationship between fine and secondarily thickened roots in these alfalfa germplasms ranges from highly branched and fibrous to strongly taprooted with few fibrous roots. Germplasms with contrasting root system architectures are being developed to serve different agronomic and environmental goals, such as rapid root elongation into the subsoil to help remediate nitrate-contaminated soil (Meyers et al., 1996), or high root length densities in the topsoil to absorb nutrients applied in agricultural and food processing wastes. Knowledge of root demography characteristics associated with these germplasms is important, because rapid root turnover could help alleviate N deficiencies in eroded soils or in neighboring plants, but also might exacerbate a nitrate contamination problem in soils subject to nitrate leaching.

Our objective in this experiment was to determine the extent and patterns of fine-diameter root production and loss of four contrasting alfalfas during the stand establishment year. Two germplasms are essentially isolines that differ in N_2 fixation and two are selections that differ in root system morphology.

Materials and methods

This study was conducted at the University of Minnesota Sand Plain Research Farm, Becker, MN. A pit (6 by 3 by 1.5 m deep) was dug in a loamy sand-textured Udorthentic Haploboroll on 18 May 1994. Pit walls were reinforced with plywood and the top protected with a low roof, pitched to the north and south. Water runoff from the roof was collected in gutters, which diverted water to a low area about 3 m from the plots. Two replicate plot areas (1.8-m long by 2.4-m wide) were located adjacent to both the north and south sides of the pit to minimize the influence of shading from the roof. Soil was fertilized according to University of Minnesota recommendations (Rehm and Schmitt, 1989).

Minirhizotron tube preparation and installation

Cellulose acetate butyrate minirhizotron tubes (1.82 m long, 57-mm o.d., and 51-mm i.d.) were marked lengthwise on two opposite sides with dots (0.8-mm diam.), 26 mm apart. These dots served as registration marks during recording. Both ends of the tubes were closed with no. 11 neoprene stoppers.

Under the central three rows of each experimental plot (eight rows total) approximately one week before planting, minirhizotron tubes (with registration marks in horizontal position) were placed horizontally into small trenches dug by hand perpendicular to the pit walls. The longitudinal rows of dots were placed at depths of 10, 20, and 40 cm from the soil surface. Observation depth was assigned randomly to three tubes under future plant rows within 1.8-m long by 1.2-m wide areas, and only one minirhizotron tube was installed under any given row. Soil was replaced manually, assuring good contact with the minirhizotron surfaces. The end of each minirhizotron tube extending into the pit was painted black to prevent entry of light. Minirhizotron tubes were stabilized by attaching them to the plywood reinforcement walls with expanding foam insulation.

Planting and harvesting

Before planting, seeds of four alfalfa germplasms were inoculated with a commercial mixture of *Rhizobium meliloti* (Nitragin, Milwaukee, Wisconsin, USA). Four alfalfa germplasms were seeded by hand (31 May through 2 June) directly over the tubes and border areas in 8 rows spaced 15 cm apart. Germplasms included: effectively nodulated 'Agate' (AGATE); 'Ineffective Agate', an ineffectively nodulated non-N_2-fixing fixing near isoline of Agate (INEFF) (Barnes et al., 1990); a germplasm having a tap root with few fibrous roots (LFTAP); and a germplasm with many fibrous roots and a strongly branch-rooted architecture (HFBRH).

The experimental design was a modified split-block with one-quarter of each 1.8-m by 2.4-m replicate planted with one of four germplasms. In relation to the pit walls for each group of three minirhizotron tubes, a germplasm was randomly assigned to the proximal 90-cm end and a different germplasm was randomly assigned to the distal 90-cm end. After emergence, seedlings were thinned to 3 cm between plants, giving a final stand of 200 plants m^{-2}. Irrigation was applied according to a modified "checkbook" method (Wright and Bergsrud, 1991). Between 21 July and 14 September, all plots received five biweekly fertilizer applications of 22 kg N ha^{-1}. Herbage was harvested by hand on 19 August and 20 October 1994.

Root samples were collected in early November by removing 3.2-cm diam. soil cores centered about 5 cm away from one plant in each plot. Roots were separated from soil in a hydropneumatic elutriator (Smucker et al., 1982). The washed roots were stained, scanned, and analyzed using a public domain software package (Dowdy et al., 1995).

Image recording and analyzing

The video-recording minirhizotron system consisted of an agricultural research color camera (Bartz Technology, Santa Barbara, CA); a high-resolution, 210-by 280-mm color monitor; a SVHS video cassette recorder; and SVHS video cassette recorder. Maximum resolution of the camera was 0.05 mm.

Beginning 22 June 1994 at the 10 and 20 cm depths, 20 minirhizotron images (13 by 18 mm) of each germplasm were recorded parallel to the soil surface (90° from vertical) on each side of the minirhizotron tubes. Recordings began 20 July at the 40 mm depth. Images were recorded from the central 52 cm of each plot to reduce the chance of including roots from the adjacent germplasm. A total of 1,920 images were acquired at each recording period (4 replicates, 4 germplasms, 3 depths, and 20 images on 2 opposite sides of a tube). Images from minirhizotron tubes were recorded biweekly until 26 October 1994.

An interactive PC-based software program (C-MAP-ROOTS, Center For Remote Sensing, Michigan State University, East Lansing, MI, USA) and an

image capture board were used to digitize each minirhizotron image from SVHS tapes. The length and width of all roots present in each image were traced using a mouse and dimensions calculated by the software were written to a relational database management software package. Roots that were visible in two consecutive images along a tube were counted as separate roots in each image. A unique identification code was assigned to each root in the database. The same code was used for each root during analysis of the same images from subsequent recording dates. Using ROOTS, identification codes, and registration marks on the minirhizotron surfaces, digitized images were recalled to overlay the tracings on the subsequent minirhizoton images. For each root, date records were kept for root appearance, secondary thickening, and death.

A root was defined as "dead" based on characteristics reflecting decomposition, that is, when at least one-third of its length was not visible or had turned black, its edges became indistinct, or it had a water-soaked appearance. Net fine root production was calculated from the difference between living and dead roots at each time of observation. Nodules were not included in fine root calculations or cohort analysis because nodules were observed infrequently in minirhizotron images (approximately 1% of all images contained nodules).

Cumulative loss of fine root length was calculated for each replicate from weighted turnover rates for the 0- to 30-cm and 30- to 60-cm increments measured with minirhizotron observations and from washed root lengths from soil cores. This approach is conservative, in that all roots present after washing the cores were considered to be alive. To our knowledge, there is no reliable way to determine whether washed roots are alive or dead. We then used specific C content (2.96 mg C m^{-1} root) and specific N content (0.22 mg N m^{-1} root) of fine roots measured in another experiment (Dubach and Russelle, 1994) to calculate total C and N loss from fine root decomposition during the stand establishment year.

From each of the three depths, three root cohorts were defined based upon three specific time periods of observations during the 1994 growing season. The selected cohorts were roots produced in early-season (between 22 June and 20 July), mid-season (between 3 and 17 August), and late-season (between 31 August and 15 September) intervals. All fine roots produced within each interval and still alive at the end of the interval were included in a given cohort. Fine roots that became secondarily thickened at a later date were excluded from cohort analysis. Survivorship curves were generated from the initial total number of roots in each cohort and the number of original members in each cohort remaining alive at subsequent biweekly observation dates up until 12 October 1994.

Statistical analyses were conducted in SAS (1990) using GLM and germplasm means were separated using Fisher's protected LSD when the F test for germplasm effects was significant ($p<0.05$). Root survivorship curves (i.e., log(fraction surviving) vs. days) were fit by linear regression, and the resulting equations were compared statistically to reduce them to a minimum set (Weisberg, 1985, pp 179–185). Apparent discontinuities in root survivorship (at 28 days in the early-season cohort and at 14 days in the mid-season cohort) were eliminated from the regressions if the external Studentized residual test was significant (Weisberg, 1985, pp 114–118).

Results

Herbage yield

At both harvests, total herbage yields were significantly lower for the INEFF cultivar than all the other germplasms (Table 1). Yields of the effectively nodulated germplasms were typical for this site.

Fine root production

The apparent diameter of fine roots increased with depth, averaging 0.36 mm at 10 cm, 0.37 mm at 20 cm, and 0.41 mm at 40 cm. Fine roots of the LFTAP germplasm were 5% thicker than roots of the other germplasms, but there was no interaction between depth and germplasm. We did not analyze root lengths seen in the minirhizotrons. Root lengths measured by washing and scanning soil cores showed no differences among germplasms nor interactions of germplasm with depth, but root length density decreased with depth (Table 2).

For all germplasms, net fine root production at the 10- and 20-cm depths rapidly increased for the first 7 to 9 weeks after planting, declined during mid-season, and then leveled off or declined slowly until October (Figure 1). The HFBRH germplasm had more net fine root production than the INEFF germplasm at the 10-cm depth at 11 weeks after planting, when all germplasms attained highest root numbers at this depth. At the 20-cm depth, the HFBRH germplasm

Table 1. Herbage dry matter yields (g m^{-2}) of four alfalfa germplasms during the establishment year on an irrigated loamy sand soil

Germplasm	Harvest 1	Harvest 2
Agate (AGATE)	243a[z]	146a
Ineffective Agate (INEFF)	113b	80b
Low fibrous, taprooted (LFTAP)	288a	155a
High fibrous, branch rooted (HFBRH)	289a	171a

[z]Means within the same depth and root condition followed by the same letter are not significantly different using Fisher's protected LSD ($p<0.05$).

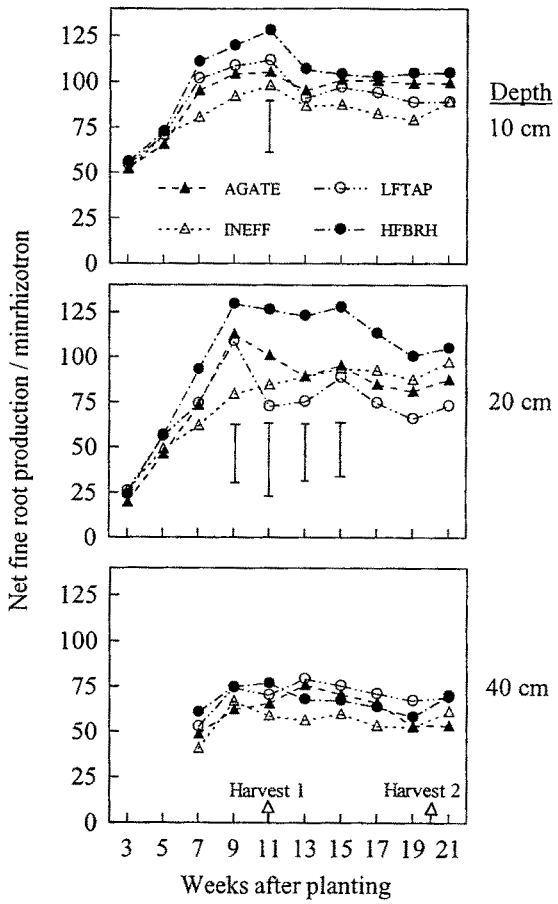

Figure 1. Net fine root production (numbers/tube) of four alfalfa germplasms at 10-, 20- and 40-cm depths. Total observed area per replicate in each case was 93.6 cm^2. For each observation date where significant differences were detected, the bar below the data points represents the Fisher's protected LSD ($p<0.05$) value. Observations at 40 cm began 4 weeks later than those at 10 and 20 cm.

again had more net fine root production than INEFF (9 and 15 weeks after planting) and more than the LFTAP germplasm (11, 13, and 15 weeks after planting). There was a sharp decline in net fine root production for the LFTAP germplasm between 9 and 11 weeks after planting, followed by a temporary increase before further declines. This decline was not due to higher fine root mortality (see discussion below), so must have been due to a large decrease in new root production. No differences were noted among germplasms for net fine root production at the 40-cm depth.

By the end of the stand establishment year, differences in root production among germplasms were evident at the 20- and 40-cm depths, but not at 10 cm (Table 3). At 20 cm, the HFBRH germplasm had significantly more living, dead and total fine roots than the LFTAP germplasm, and more dead and total fine roots than the other germplasms. At 40 cm, AGATE had fewer living fine roots than LFTAP and HFBRH, INEFF had fewer dead fine roots than HFBRH, and both AGATE and INEFF had fewer total fine roots than HFBRH. LFTAP and HFBRH had similar numbers of fine roots at this depth, in contrast to 20 cm.

Numbers of secondarily thickened roots at the season's end were similar among germplasms at the 10- and 20-cm depths, but the INEFF germplasm had fewer than the AGATE and HFBRH germplasms at 40 cm. An average of 7% of the fine roots developed into secondarily thickened roots, with no differences among germplasms or depths.

Based on estimated loss of fine root length in the upper 60 cm of soil (totaling 274,000 km ha^{-1}) and assumed C and N contents per unit root length (from Dubach and Russelle, 1994), we estimate that fine root turnover in these alfalfa germplasms released an average of about 800 kg C and 60 kg N ha^{-1} (Table 2).

Table 2. Root length, average root turnover rate, and calculated loss of C and N from root turnover in the establishment year of alfalfa in an irrigated loamy sand soil

Depth increment (cm)	Root length[w] Total (km m^{-2})	Fine[x] (km m^{-2})	Root turnover rate[y] (%)	Calculated loss of fine root[z] Length (km m^{-2})	C (g m^{-2})	N (g m^{-2})
0-10	13.7	12.7	48	11.7	35	2.6
10-20	10.9	10.1	48	9.3	28	2.0
20-30	3.3	3.1	48	2.9	9	0.6
30-40	3.0	2.8	36	1.6	5	0.4
40-60	3.6	3.3	36	1.9	6	0.4

[w]Root length density determined on roots washed from soil cores taken in early November 1994.
[x]Assumed that 7% of total root length was due to secondarily thickened roots.
[y]Average rate based on data in Table 3.
[z] Specific C content = 2.96 g km^{-1} and specific N content = 0.22 g km^{-1} (Dubach and Russelle, 1994).

Table 3. Total number of living and dead fine roots, secondarily thickened roots, and total roots observed by the end of the establishment season (26 October 1994) in four alfalfa germplasms at 10-, 20-, and 40-cm depths of an irrigated loamy sand soil. Values are the total root counts along both sides of a minirhizotron tube (52 cm total image length by 1.8 cm height)

Germplasm	Alive fine roots	Dead fine roots	Secondarily thickened roots	Total roots
10 cm depth				
AGATE	100a[z]	89a	14a	202a
INEFF	89a	96a	13a	197a
LFTAP	89a	85a	16a	189a
HFBRH	105a	94a	14a	214a
20 cm depth				
AGATE	88ab	78b	12a	177b
INEFF	97ab	68b	13a	178b
LFTAP	73b	75b	12a	161b
HFBRH	105a	101a	13a	219a
40 cm depth				
AGATE	54b	38ab	8a	100b
INEFF	61ab	28b	3b	92b
LFTAP	69a	36ab	5ab	110ab
HFBRH	70a	42a	8a	120a

[z]Means within the same depth and root condition followed by the same letter are not significantly different using Fisher's protected LSD (p<0.05).

Cohort analysis

Only five rates of exponential decline were required to explain over 95% of the variation in all root survivorship curves (Figure 2). All of these equations had the form ln$y$ = 0.00350 + b(days), and thus, all had y intercept = 0.996. Slope coefficients (b) were -0.00533, -0.00760, -0.00952, -0.01208, and -0.01487, resulting in estimated median fine root life spans (±SE) of 131

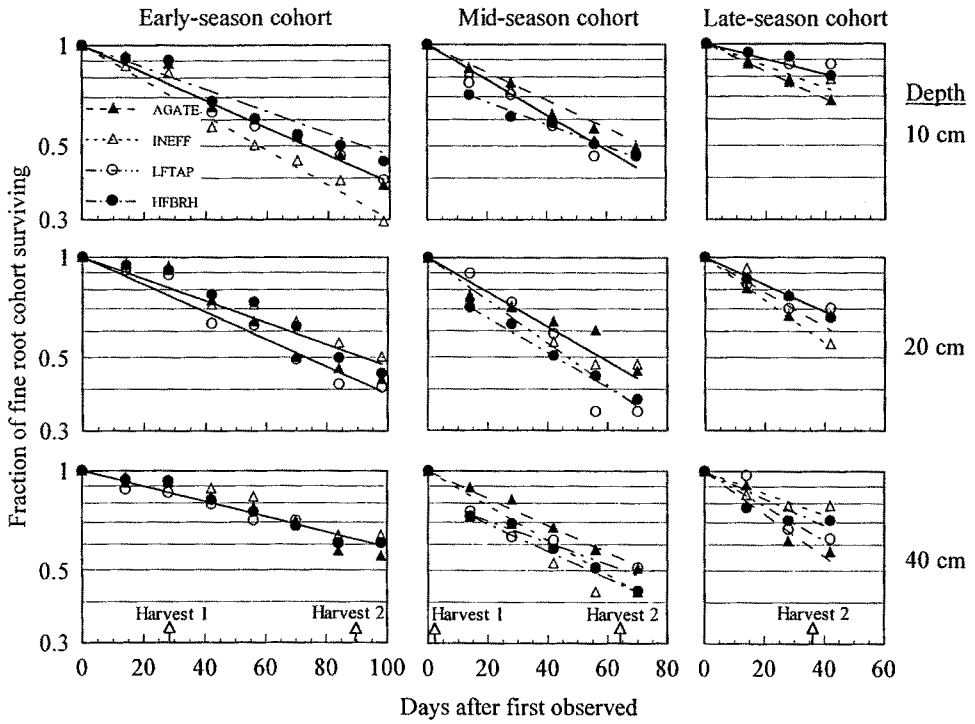

Figure 2. Fraction of surviving fine roots in three contemporaneous cohorts at 10-, 20-, and 40-cm depths. The period of observation extends to 26 October 1994 in each case. Five slopes ($\log y = a + bx$) explained 95% of the variation among survivorship curves, all with y intercept = 1, except for four truncated curves in the mid-season cohort (see text for explanation). Solid lines are used where more than one germplasm fit a given equation. Early season cohorts at 40 cm include observations from 20 July only. Note the y axis is plotted on a $\log_{10}$ scale.

± 5, 92 ± 3,73 ± 2, 58 ± 2, and 47 ± 2 days, respectively.

In the early-season cohorts, apparent fine root life span increased as depth increased, i.e. slopes became less negative (Figure 2). There was a significant reduction in fine root mortality in the two weeks before harvest at the 10-cm depth, according to the external Studentized residual test. This was followed by a pronounced increase in mortality following herbage removal (24% loss in fine roots), and then a return to mortality rates similar to those at the beginning of the observation period. A similar decline in fine root mortality before, and increased mortality after, harvest occurred at 20 cm in three germplasms, but not in HFBRH. In contrast, only the HFBRH germplasm showed significant deviation in fine root survival of the early season cohort at 40 cm near the time of first harvest. These results demonstrate that root mortality was altered near the time of first herbage harvest, and as a result, data points at 28 days were omitted from all regressions at the 10-cm depth, from three at 20 cm, and from one at 40cm, because they were deemed to be 'outliers.' In contrast, there was no statistically significant deviation from the regressions in the samples taken near harvest 2.

The INEFF germplasm had lower fine root survival in the early-season cohort at 10 cm than N_2-fixing germplasms (median life span = 58 days), and survival of fine roots in HFBRH (median life span = 92 days) was greater than in LFTAP and AGATE (median life span = 73 days). At 20 cm, fine root survival was similar for INEFF and HFBRH (median life span = 92 days) and superior to AGATE and LFTAP (median life span = 73 days). All germplasms had similar root survivorship curves at 40 cm, with an estimated median fine root life span of about 131 days.

In the mid-season cohorts, fine roots of HFBRH at all depths had more rapid rates of root mortality immediately after the first herbage harvest than later (average 29% loss over 2 weeks, Figure 2). This also was true for LFTAP roots at 40 cm, but, unlike the early-season cohort, did not occur in other depth-germplasm combinations. Mortality rates generally were more rapid in the mid- than early-season cohort of all germplasms

at 20 and 40 cm, but were similar at 10 cm. Ignoring the impact of initial root mortality after harvest 1, fine root survivorship generally was smallest for INEFF and LFTAP at 10 cm, LFTAP at 20 cm, and INEFF at 40 cm. Median fine root life spans ranged from 47 to 92 days for the mid-season cohort. As was the case in the early-season cohort, there was no evidence of increased fine root survival before the second harvest.

Only four observation times were available for the late-season cohort, making conclusions less robust. Regression analysis revealed that fine root median life spans tended to be longer at 10 cm (73 to 131 days) than earlier cohorts, but shorter or equal at deeper depths (47 to 73 days at 20 cm; 7 to 92 days at 40 cm). Consistent with the early- and mid-season cohorts, there was no statistically significant evidence that roots at any depth survived longer in the period immediately before harvest than earlier.

Discussion

The lower yield attained by the INEFF cultivar was expected, due to low soil N mineralization rate at this site (Thicke et al., 1993). Unlike the other germplasms, this cultivar nodulates but does not have the capacity to obtain N from symbiotic N_2 fixation. Herbage yields of the INEFF cultivar can equal those of AGATE when sufficient inorganic N is supplied on this loamy sand soil (Lamb et al., 1995b). We applied a total of 110 kg N ha^{-1} in fertilizer during the summer, which evidently was insufficient to offset the lack of N_2 fixation.

Lower herbage yield of the INEFF germplasm was not reflected in lower numbers of living or total fine roots produced by the end of the season. Similarly, other work at this location found no differences in total root mass, fine root mass, or fine root length between ineffective and effective alfalfa germplasms, despite large differences in herbage yield (Blumenthal and Russelle, 1996; Lory et al., 1992). This result suggests that the primary limiting factor for growth at this location was N supply. Other non-N_2-fixing species partition more dry mass to roots than shoots under low N supply (Boot and Mensink, 1991; Ryser and Lambers, 1995), because N deficits retard the initiation and expansion of new leaves.

Some researchers have hypothesized that root turnover may decrease under conditions of limiting nutrient availability, so that the plant will maintain a long-lived extensive root biomass for nutrient acquisition (Cheng et al., 1990; Nadelhoffer et al., 1985) INEFF had a faster fine root mortality rate than AGATE in four depth-cohort combinations, an equal mortality rate in two cases, and a slower rate in three combinations. Because these alfalfa germplasms are similar except for their ability to fix N_2 symbiotically, our data do not support the idea of either increased or decreased root longevity under N deficiency in the first year of alfalfa growth.

Mortality rate of the early cohort of fine roots decreased markedly during the 2 weeks before the first herbage harvest in mid-August. Unfortunately, we made no observations of plant development stage. Lack of a similar decline in fine root loss before the second harvest raises the question as to whether this observation is due to particular conditions during this trial or is typical only for alfalfa that has attained some minimum developmental stage. The crop had visible symptoms of potato leafhopper (*Empoasca fabae*) infestation before the first harvest, which may have affected our observations, because basipetal photosynthate transport via phloem is blocked at their feeding sites (Stuteville and Erwin, 1990). Little net root production occurred during the 2 weeks before harvest 1 (Figure 1), which indicates that few fine roots were being generated during this period, since loss rate also was low (Figure 2). This hiatus in both root production and loss implies a general root system response to the particular conditions or developmental stage.

After herbage removal, a rapid increase in fine root mortality occurred in both the early and mid-season cohorts at depths of 10 and 20 cm. Decreased cohort survival may have resulted from smaller C allocation from the shoot to roots, to increased respiration in response to stress and regrowth (Heichel et al., 1988), or to the combination. Meyerhoff (1981) estimated that fibrous root mass turnover was 23% of that present before harvest in an established alfalfa crop. Our minirhizotron observations confirm Meyerhoff's (1981) results, because there was an average 22% decline in the early-season cohorts at 10 and 20 cm during the two weeks post harvest (Figure 2). We saw a similar decline (29%) at all depths in the mid-season cohort only in the germplasm selected for fibrous rootedness, and only at 40 cm for the taprooted germplasm (Figure 2).

Does root system architecture alter root demography? Based on minirhizotron observations, which sample the root system more intensively than soil cores, there was a clear tendency for the HFBRH germplasm to produce more fine roots at the 20-cm depth than the LFTAP germplasm (Figure 1). This is the first

independent evidence that selection for large numbers of lateral and fibrous roots causes a corresponding increase in root counts. Plant selection and evaluation during breeding were based on traits in the upper 20 to 30 cm of the root zone of excavated plants (J F S Lamb, pers. commun., 1995). However, at 10 and 40 cm, our data indicate that root numbers were similar between LFTAP and HFBRH, and no differences were detected in root length densities of washed roots sampled at the end of the establishment year. These two germplasms also had similar numbers of secondarily thickened roots. We did not find differences in total fine root turnover rates between the HFBRH and LFTAP germplasms, although our analysis of cohort survival demonstrated that a few temporal differences in root mortality rate occurred.

Thus, although overall fine root survival during the stand establishment year was not influenced by root system architecture, patterns of fine root mortality did differ during the season. Selection for high vs. low fibrous root mass in the upper part of the root zone may not change other zones of the root system, nor necessarily alter seasonal total root turnover rate. However, further evaluation of temporal patterns in root mortality of these germplasms is warranted, because these patterns will influence the timing of N release from the decomposing roots.

Our estimate of N loss in this experiment (60 kg N ha^{-1}) is considerably larger than the 21 kg N ha^{-1} estimated at the end of the establishment year for 'Saranac' alfalfa (Dubach and Russelle, 1994) and 32 kg N ha^{-1} yr^{-1} in older alfalfa stands in Sweden (Andrén et al., 1990). This was not due to root turnover rate, as our averages are similar to those of Dubach and Russelle (1994). We attribute the high estimate of N loss to the large root length densities measured here, which are significantly higher than those found in most other reports (Blumethal and Russelle, 1996; Dubach and Russelle, 1994; Grimes et al., 1978; Shein and Pachepsky, 1995). These studies reported total root length densities of between 5.1 and 8.2 cm cm^{-3} for the upper 10 to 17.5 cm, in contrast to the 13.7 cm cm^{-3} found in this experiment. Our root length measurements are similar to those of Hancock (1985), who found total fine root length densities in the upper 15 cm of soil ranging from about 5.0 to 13.4 cm cm^{-3} in commercial alfalfa fields in the Central Valley of California. Nevertheless, we suspect that our estimates of root length density may be exaggerated because we sampled close to the plant crowns. This may be a region of higher than average root length density, but we have no evidence to support this speculation.

In contrast to what may be overestimates of N loss from fine root decomposition, total C loss from fine root mortality was estimated to be about 800 kg C ha^{-1} in this experiment (Table 2), about one-half the estimate of Andrén et al. (1990) for established alfalfa. Both estimates are underestimates of total C deposition, because both are based on C content of roots and neither includes C addition from exudates and cell sloughing, which often equals C input in root biomass in other species (Swinnen et al., 1995).

Herbage harvest appeared to reduce cohort survival during mid-season. Our results show that root turnover in the 2 weeks following herbage harvest may release C and N into the soil at a faster rate than during other times during the growing season. Similar effects are likely with herbage removal by close grazing, and might occur if herbage is damaged by insects or disease. We speculate that in mixed stands, shading by a taller species also may promote root turnover in alfalfa, as it does in other legumes (Butler et al., 1959). More pronounced root turnover in alfalfa should promote accumulation of organic C and N in the soil, and may improve transfer of fixed N to neighboring plants.

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